CODING FORMS FOR SRC INDEXING

Microfiche No.	
OTS0555855	

New Doc ID	Old Doc ID
88-920010586	8EHQ-1092-12377

Date Produced	Date Received	TSCA Section
04/16/74	10/27/92	8ECP

Submitting Organization
E I DUPONT DE NEMOURS & CO

Contractor
HASKELL LABORATORY

Document Title
INITIAL SUBMISSION: IMMUNOPATHOLOGICAL FEATURES OF ISOCYANATE COMPOUNDS IN GUINEA PIGS WITH COVER LETTER DATED 10/15/92

Chemical Category
TOLUENE 2,4-DIISOCYANATE, METHYLENEBISPHENYL ISOCYANATE, *

8(e)

CAP

(COMPLIANCE AUDIT PROGRAM)

12377

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LG-4878 REV. 9/89

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Document Processing Center (TS-790)
Office of Pollution Prevention and Toxics
Environmental Protection Agency
401 M Street., S.W.
Washington, D.C. 20460
Attn: Section 8(e) Coordinator (CAP Agreement)

Dear Coordinator:

8ECAP-0025

On behalf of the Regulatee and pursuant to Unit II B.1.b. and Unit II C of the 6/28/91CAP Agreement, E.I. Du Pont de Nemours and Co. hereby submits (*in triplicate*) the attached studies. Submission of this information is voluntary and is occasioned by unilateral changes in EPA's standard as to what EPA now considers as reportable information. Regulatee's submission of information is made solely in response to the new EPA §8(e) reporting standards and is not an admission: (1) of TSCA violation or liability; (2) that Regulatee's activities with the study compounds reasonably support a conclusion of substantial health or environmental risk or (3) that the studies themselves reasonably support a conclusion of substantial health or environmental risk.

The "Reporting Guide" creates new TSCA 8(e) reporting criteria which were not previously announced by EPA in its 1978 Statement of Interpretation and Enforcement Policy, 43 Fed Reg 11110 (March 16, 1978). The "Reporting Guide states criteria which expands upon and conflicts with the 1978 Statement of Interpretation. Absent amendment of the Statement of Interpretation, the informal issuance of the "Reporting Guide" raises significant due processes issues and clouds the appropriate reporting standard by which regulated persons can assure TSCA Section 8(e) compliance.

For Regulatee,

Mark H. Christman
Counsel
Legal D-7158
1007 Market Street
Wilmington, DE 19898
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ATTACHMENT 1

Submission of information is made under the 6/28/91 CAP Agreement, Unit II. This submission is made voluntarily and is occasioned by recent changes in EPA's TSCA §8(e) reporting standard; such changes made, for the first time in 1991 and 1992 without prior notice and in violation of Regulatee's constitutional due process rights. Regulatee's submission of information under this changed standard is not a waiver of its due process rights; an admission of TSCA violation or liability, or an admission that Regulatee's activities with the study compounds reasonably support a conclusion of substantial risk to health or to the environment. Regulatee has historically relied in good faith upon the 1978 Statement of Interpretation and Enforcement Policy criteria for determining whether study information is reportable under TSCA §8(e), 43 Fed Reg 11110 (March 16, 1978). EPA has not, to date, amended this Statement of Interpretation.

After CAP registration, EPA provided the Regulatee the June 1, 1991 "TSCA Section 8(e) Reporting Guide". This "Guide" has been further amended by EPA, EPA letter, April 10, 1992. EPA has not indicated that the "Reporting Guide" or the April 1992 amendment supersedes the 1978 Statement of Interpretation. The "Reporting Guide" and April 1992 amendment substantively lowers the Statement of Interpretation 's TSCA §8(e) reporting standard[2]. This is particularly troublesome as the "Reporting Guide" states criteria, applied retroactively, which expands upon and conflicts with the Statement of Interpretation.[3] Absent amendment of the Statement of Interpretation, the informal issuance of the "Reporting Guide" and the April 1992 amendment clouds the appropriate standard by which regulated persons must assess information for purposes of TSCA §8(e).

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[2]In sharp contrast to the Agency's 1977 and 1978 actions to soliciting public comment on the proposed and final §8(e) Policy, EPA has unilaterally pronounced §8(e) substantive reporting criteria in the 1991 Section 8(e) Guide without public notice and comment, See 42 Fed Reg 45362 (9/9/77), "Notification of Substantial Risk under Section 8(e): Proposed Guidance".

[3]A comparison of the 1978 Statement of Interpretation and the 1992 "Reporting Guide" is a appended.

Throughout the CAP, EPA has mischaracterized the 1991 guidance as reflecting "longstanding" EPA policy concerning the standards by which toxicity information should be reviewed for purposes of §8(e) compliance. Regulatee recognizes that experience with the 1978 Statement of Interpretation may cause a review of its criteri. Regulatee supports and has no objection to the Agency's amending reporting criteria *provided that* such amendment is not applied to the regulated community in an unfair way. However, with the unilateral announcement of the CAP under the auspices of an OCM enforcement proceeding, EPA has wrought a terrific unfairness since much of the criteria EPA has espoused in the June 1991 Reporting Guide and in the Agency's April 2, 1992 amendment is new criteria which does not exist in the 1978 Statement of Interpretation and Enforcement Policy.

The following examples of new criteria contained in the "Reporting Guide" that is not contained in the Statement of Interpretation follow:

- o even though EPA expressly disclaims each "status report" as being preliminary evaluations that should not be regarded as final EPA policy or intent[4], the "Reporting Guide" gives the "status reports" great weight as "sound and adequate basis" from which to determine mandatory reporting obligations. ("Guide" at page 20).
- o the "Reporting Guide" contains a matrix that establishes new numerical reporting "cutoff" concentrations for acute lethality information ("Guide" at p. 31). Neither this matrix nor the cutoff values therein are contained in the Statement of Interpretation. The regulated community was not made aware of these cutoff values prior to issuance of the "Reporting Guide" in June, 1991.
- othe "Reporting Guide" states new specific definitional criteria with which the Agency, for the first time, defines as 'distinguishable neurotoxicological effects'; such criteria/guidance not expressed in the 1978 Statement of Interpretation.[5],

- othe "Reporting Guide" provides new review/ reporting criteria for irritation and sensitization studies; such criteria not previously found in the 1978 Statement of Interpretation/Enforcement Policy.
- othe "Reporting Guide" publicizes certain EPA Q/A criteria issued to the Monsanto Co. in 1989 which are not in the Statement of Interpretation; have never been published in the Federal Register or distributed by the EPA to the Regulatee. Such Q/A establishes new reporting criteria not previously found in the 1978 Statement of Interpretation/Enforcement Policy .

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[4]The 'status reports' address the significance, if any, of particular information reported to the Agency, rather than stating EPA's interpretation of §8(e) .eporting criteria. In the infrequent instances in which the status reports contain discussion of reportability, the analysis is invariably quite limited, without substantial supporting scientific or legal rationale.

[5] *See, e.g,* 10/2/91 letter from Du Pont to EPA regarding the definition of 'serious and prolonged effects' as this term may relate to transient anesthetic effects observed at lethal levels; 10/1/91 letter from the American Petroleum Institute to EPA regarding clarification of the Reporting Guide criteria.

In discharging its responsibilities, an administrative agency must give the regulated community fair and adequate warning to as what constitutes noncompliance for which penalties may be assessed.

> Among the myriad applications of the due process clause is the fundamental principle that statutes and regulations which purport to govern conduct must give an adequate warning of what they command or forbid.... Even a regulation which governs purely economic or commercial activities, if its violation can engender penalties, must be so framed as to provide a constitutionally adequate warning to those whose activities are governed.

Diebold, Inc. v. Marshall, 585 F.2d 1327, 1335-36 (D.C. Cir. 1978). *See also*, Rollins Environemntal Services (NJ) Inc. v. U.S. Environmental Protection Agency, 937 F. 2d 649 (D.C. Cir. 1991).

While neither the are rules, This principle has been applied to hold that agency 'clarification', such as the Statement of Interpretation, the "Reporting Guide" nor the April 1992 amendments will not applied retroactively.

> ...a federal court will not retroactively apply an unforeseeable interpretation of an administrative regulation to the detriment of a regulated party on the theory that the post hoc interpretation asserted by the Agency is generally consistent with the policies underlying the Agency's regulatory program, when the semantic meaning of the regulations, as previously drafted and construed by the appropriate agency, does not support the interpretation which that agency urges upon the court.

Standard Oil Co. v. Federal Energy Administration, 453 F. Supp. 203, 240 (N.D. Ohio 1978), aff'd sub nom. Standard Oil Co. v. Department of Energy, 596 F.2d 1029 (Em. App. 1978):

The 1978 Statement of Interpretation does not provide adequate notice of, and indeed conflicts with, the Agency's current position at §8(e) requires reporting of all 'positive' toxicological findings without regard to an assessment of their relevance to human health. In accordance with the statute, EPA's 1978 Statement of Interpretation requires the regulated community to use scientific judgment to evaluate the significance of toxicological findings and to determining whether they reasonably support a conclusion of a substantial risk. Part V of the Statement of Interpretation urges persons to consider "the fact or probability" of an effect's occurrence. Similarly, the 1978 Statement of Interpretation stresses that an animal study is reportable only when "it contains reliable evidence ascribing the effect to the chemical." 43 Fed Reg. at 11112. Moreover, EPA's Statement of Interpretation defines the substantiality of risk as a function of both the seriousness of the effect and the probability of its occurrence. 43 Fed Reg 11110 (1978). Earlier Agency interpretation also emphasized the "substantial" nature of a §8(e) determination. See 42 Fed Reg 45362, 45363

(1977). [Section 8(e) findings require "extraordinary exposure to a chemical substance...which critically imperil human health or the environment"].

The recently issued "Reporting Guide" and April 1992 Amendment guidance requires reporting beyond and inconsistent with that required by the Statement of Interpretation. Given the statute and the Statement of Interpretation's explicit focus on substantial human or environmental risk, whether a substance poses a "substantial risk" of injury requires the application of scientific judgment to the available data on a case-by-case basis.

If an overall weight-of-evidence analysis indicates that this classification is unwarranted, reporting should be unnecessary under §8(e) because the available data will not "reasonably support the conclusion" that the chemical presents a substantial risk of serious adverse consequences to human health.

Neither the legislative history of §8(e) nor the plain meaning of the statute support EPA's recent lowering of the reporting threshold that TSCA §8(e) was intended to be a sweeping information gathering mechanism. In introducing the new version of the toxic substances legislation, Representative Eckhart included for the record discussion of the specific changes from the version of H. R. 10318 reported by the Consumer Protection and Finance Subcommittee in December 1975. One of these changes was to modify the standard for reporting under §8(e). The standard in the House version was changed from "causes or contributes to an unreasonable risk" to "causes or significantly contributes to a substantial risk". This particular change was one of several made in TSCA §8 to avoid placing an undue burden on the regulated community. The final changes to focus the scope of Section 8(e) were made in the version reported by the Conference Committee.

The word "substantial" means "considerable in importance, value, degree, amount or extent". Therefore, as generally understood, a "substantial risk" is one which will affect a considerable number of people or portion of the environment, will cause serious injury and is based on reasonably sound scientific analysis or data. Support for the interpretation can be found in a similar provision in the Consumer Product Safety Act. Section 15 of the CPSA defines a "substantial product hazard" to be:

> "a product defect which because of the pattern of defect, the number of defective products distributed in commerce, the severity of the risk, or otherwise, creates a substantial risk of injury to the public."

Similarly, EPA has interpreted the word 'substantial' as a quantitative measurement. Thus, a 'substantial risk' is a risk that can be quantified, *See*, 56 Fed Reg 32292, 32297 (7/15/91). Finally, since information pertinent to the exposure of humans or the environment to chemical substances or mixtures may be obtained by EPA through Sections 8(a) and 8(d) regardless of the degree of potential risk, §8(e) has specialized function. Consequently, information subject to §8(e) reporting should be of a type which would lead a reasonable man to conclude that some type action was required immediately to prevent injury to health or the environment.

Attachment

Comparison:

R orting triggers found in the 1978 "Statement of Interpretation/ Enforcement Policy",43 Fed Reg 11110 (3/16/78) and the June 1991 *Section 8(e) Guide.*

TEST TYPE	1978 POLICY CRITERIA EXIST?	New 1991 GUIDE CRITERIA EXIST?
ACUTE LETHALITY		
Oral	N}	Y}
Dermal	N}	Y}
Inhalation (Vapors)	}[6]	}[7]
aerosol	N}	Y}
dusts/ particles	N}	Y}
SKIN IRRITATION	N	Y[8]
SKIN SENSITIZATION (ANIMALS)	N	Y[9]
EYE IRRITATION	N	Y[10]
SUBCHRONIC (ORAL/DERMAL/INHALATION)	N	Y[11]
REPRODUCTION STUDY	N	Y[12]
DEVELOPMENTAL TOX	Y[13]	Y[14]

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[6] 43 Fed Reg at 11114, comment 14:
"This policy statements directs the reporitng of specifiec effects when unknown to the Administrator. Many routine tests are based on a knowledge of toxicity associated with a chemicalL unknown effects occurring during such a range test may have to be reported if they are those of concern tot he Agency and if the information meets the criteria set forth in Parts V and VII."

[7] Guide at pp.22, 29-31.

[8] Guide at pp-34-36.

[9] Guide at pp-34-36.

[10] Guide at pp-34-36.

[11] Guide at pp-22; 36-37.

[12] Guide at pp-22

[13] 43 Fed Reg at 11112
"Birth Defects" listed.

[14] Guide at pp-22

NEUROTOXICITY	N	Y[15]
CARCINOGENICITY	Y[16]	Y[17]
MUTAGENICITY		
In Vitro	Y}[18]	Y} [19]
In Vivo	Y}	Y}
ENVIRONMENTAL		
Bioaccumulation	Y}	N
Bioconcentration	Y}[20]	N
Oct/water Part. Coeff.	Y}	N
Acute Fish	N	N
Acute Daphnia	N	N
Subchronic Fish	N	N
Subchronic Daphnia	N	N
Chronic Fish	N	N
AVIAN		
Acute	N	N
Reproductive	N	N
Reprodcutive	N	N

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[15] Guide at pp-23; 33-34.

[16] 43 Fed Reg at 11112
"Cancer" listed

[17] Guide at pp-21.

[18] 43 Fed Reg at 11112; 11115 at Comment 15
"Mutagenicity" listed/ *in vivo* vs *invitro* discussed; discussion of "Ames test".

[19] Guide at pp-23.

[20] 43 Fed Reg at 11112; 11115 at Comment 16.

CAS # 584-84-9; 26447-40-5; 9016-87-9; 5124-30-1

Chem: Toluene 2,4-diisocyanate (TDI); methylenebisphenyl isocyanate (MDI); polymethylene polyphenylisocyanate (PAPI); and 4,4-methylenebiscyclohexyl isocyanate

Title: Immunopathological features of isocyanate compounds

Date: 4/16/74

Summary of Effects: depression; incoordinaton, hyperactivity

IMMUNOPATHOLOGICAL FEATURES OF ISOCYANATE COMPOUNDS

Haskell Laboratory Report No. 249-74

Medical Research Project No. 10-C-5

Report by: K. P. Lee

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Date: April 16, 1974

IMMUNOPATHOLOGICAL FEATURES OF ISOCYANATE COMPOUNDS

Haskell Laboratory Report No. 249-74

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IMMUNOPATHOLOGICAL FEATURES OF ISOCYANATE COMPOUNDS

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Medical Research Project No. 10-C-5

The aerosol exposure and measurements of airway resistance were performed by Miss Rhoda M. Brown under the direction of Dr. Franklin D. Griffith. The passive cutaneous anaphylaxis and gel diffusion tests were carried out by Mr. Francis L. Ulmer and Mr. William I. Swan under the direction of Dr. Ki Poong Lee. Gross pathology was carried out by Mr. August H. Stenholm, Mr. William I. Swan and Mr. Francis L. Ulmer under the supervision of Dr. Rudolf Culik and Dr. James G. Aftosmis. Microscopic slides were prepared by Mrs. Jean A. Hostetler, Mr. Anthony T. DiLorenzo and Mrs. Joan A. Dimeler under the direction of Dr. Ki Poong Lee. Histopathologic evaluation of the tissues was conducted by Dr. Ki Poong Lee.

IMMUNOPATHOLOGICAL FEATURES OF ISOCYANATE COMPOUNDS

Haskell Laboratory Report No. 249-74

Medical Research Project No. 10-C-5

SUMMARY

The causal relationship between airway resistance and pathological changes, as well as immunological response, was examined for guinea pigs following consecutive aerosol exposure to toluene 2,4-diisocyanate (TDI), methylenebisphenyl isocyanate (MDI), polymethylene polyphenylisocyanate (PAPI®*) and 4,4-methylenebiscyclohexyl isocyanate (Hylene® W). Serum antibodies were detected by passive cutaneous anaphylaxis (PCA) test in the sera from guinea pigs exposed to TDI but other isocyanates failed to produce positive results when tested at lower concentrations. Significant elevation of airway resistance in response to a single challenge exposure to TDI was obtained after consecutive sensitization exposures, suggesting an asthma-like response. Challenge to PAPI® produced slight airway resistance; other isocyanates failed to elicit airway resistance following challenge at lower concentrations. Guinea pigs exposed to TDI revealed prominent exudate consisting mainly of PMN (polymorphonuclear) leucocytes in the airways and it superimposed to obliterative bronchiolitis. Prominent obliterative bronchiolitis with precipitate of inhaled material was found in the guinea pigs exposed to PAPI®. Other isocyanates induced slight tracheobronchitis.

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* Registered trademark of Upjohn Company for polymethylene polyphenylisocyanate.

IMMUNOPATHOLOGICAL FEATURES OF ISOCYANATE COMPOUNDS

Haskell Laboratory Report No. 249-74

Medical Research Project No. 10-C-5

INTRODUCTION

Isocyanates are widely used today in the production of polyurethane foam, paints, lacquers, adhesives, and insulating materials. Zapp described in detail the toxicological and industrial aspects of isocyanates (76). Isocyanate vapor, which is liberated during the production of these products, has been known to cause irritation of skin, mucous membranes of the conjunctiva, and respiratory tracts of animals and humans (10, 32, 43, 75). In addition, there were reports indicating extrinsic asthma-like reactions in workers who were exposed repeatedly to low concentrations of these compounds (34, 65, 69, 75). Respiratory hypersensitivity creates a serious problem among workers because hypersensitive persons become unable to work. Cumulative effects of toluene 2,4'-diisocyanate (TDI) on the lung and decreased pulmonary function were found among symptomatic and asymptomatic workers (56, 59).

Important questions have not been fully explained for many years as to whether asthmatic symptoms and pulmonary lesions resulting from isocyanate exposure were related to immunological reactions or to direct chemical irritation. The purpose of this study is an attempt to determine immunogenicity as well as cross-antigenicity of isocyanates, and furthermore, causal relationships between pathological changes of the lung and immunological response.

MATERIALS AND METHODS

Aerosol Exposure

After a series of inhalation tests for guinea pigs with various concentrations of the isocyanates to determine tolerable concentrations for consecutive sensitization exposures, 30 male albino guinea pigs were divided into five equal groups and exposed as follows:

Group 1 - Served as control and exposed to air.

Group 2 - Exposed to TDI at an average of 60.7 µg/L during sensitization and 9.3 µg/L for a single challenge exposure.

MATERIALS AND METHODS (Continued)

Aerosol Exposure (Continued)

Group 3 - Exposed to an average of 4.63 µg/L of polyethylene-polyphenylisocyanate (PAPI®*) during sensitization and 4.60 µg/L for a single challenge exposure.

Group 4 - Exposed to an average of 0.94 µg/L of methylenebisphenyl isocyanate (MDI) during sensitization and 2.5 µg/L for a single challenge exposure.

Group 5 - Exposed to an average of 6.70 µg/L of 4,4-methylenebis-cyclohexyl isocyanate (Hylene® W) as the sensitizing exposure and 1.60 µg/L for a single challenge exposure.

Isocyanate aerosols were obtained by heating at 75-120°C in an apparatus as shown in Figure 1. Dry house-air was metered into a round-bottomed flask containing the isocyanate through a stainless steel nebulizer. The aerosol passed through a glass side-arm delivery tube with an air dilution port into an 18-liter bell jar which was used as an exposure chamber. Samples of the chamber atmosphere were taken at least three times during each exposure and analyzed by using the colorimetric method (37, 45). As sensitizing exposures, one group of six guinea pigs were exposed to each isocyanate four hours per day for five days. Subsequently, two weeks after the last sensitizing exposure, the guinea pigs received a single challenge exposure for four hours. Three animals were sacrificed immediately after the challenge exposure and another three animals were killed seven days later for pathological examination. Blood was collected from the heart at the time of the sacrifice in order to obtain anti-isocyanate immune serums for PCA and the gel diffusion test. Lungs were fixed by infusion with Bouin's solution and prepared for microscopic slides.

PASSIVE CUTANEOUS ANAPHYLAXIS (PCA)

Methods based on a report of Ovary were used (51). A series of titration tests were performed on the skin of guinea pigs using various concentrations of the isocyanate compounds in order to determine proper concentrations for a challenge dose and to avoid non-specific reaction. Six test sites were prepared on the dorsal surface of the skin of a guinea pig, after shaving the fur 24 hours prior to intradermal injection of the antisera. Guinea pigs were passively sensitized by intradermal injection with 0.1 ml of antisera. After a latent period of 24 hours, 0.5 to 1 ml of 1% Evan's blue dye in saline was injected intracardially and then 0.5 ml of 1% TDI, 1% MDI, 1% PAPI® and 0.1% Hylene® W in dioxane were applied topically on the sites injected with the antisera. The site injected with TDI antiserum was used as a positive control and normal serum was used as a negative control. Also, sites where isocyanate alone was applied were used as negative controls. In addition to guinea pig anti-isocyanate sera, human sera from five subjects, clinically suspected to be hypersensitive to TDI, were subjected to this test.

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* Registered trademark of Upjohn Company for polymethylene polyphenyl-isocyanate.

IMMUNIZATION

In order to obtain immune antisera for isocyanates, one group of rabbits and guinea pigs were immunized by daily topical skin application with 0.5 ml of 1% isocyanate in dioxane to the shaved back for two weeks. Another group of animals received intramuscular injections for 10 days with 0.2 ml of isocyanate antigen which was prepared by mixing 1 ml of 10% isocyanates with 10 ml of incomplete Freund's adjuvant.

GEL-DIFFUSION TEST

The gel-diffusion test was based on the procedures described by Ouchterlony (50) and Lee and Olson (40). TDI-bovine serum albumin (BSA) conjugate was prepared by a modification of Campbell (16).

PY FUNCTION TEST

Airway resistance was measured by a modification of the method described by Amdur and Mead (1).

RESULTS

Clinical Observation

During sensitization and challenge exposure to TDI, the guinea pigs revealed respiratory difficulty, i.e.,mouth breathing, or gasping for air. The animals also showed general discomfort such as hyperemic ears, hyperactivity with subsequent depression, lacrimation, nasal discharge, cyanosis and incoordination. In comparison with the TDI exposures, animals exposed to MDI, PAPI® and Hylene® W developed mild respiratory distress, hyperactivity and ear hyperemia. At the beginning of each exposure, the control group was excited and then became restless throughout the exposure period. The blood cell count revealed normal limits of eosinophil number, but heterophil number was increased moderately in all of the animals following exposure to the isocyanates.

Airway Resistance

The results of the airway resistance measurements during the control, sensitization, recovery, and challenge exposures with the four isocyanates are illustrated in Figure 2 and Table I. The average of the measurements made on the control animals was used as the control baseline for comparing each group's response. During the sensitization period, the animals exposed to TDI developed steep stepwise increases in airway resistance and attained values approximately three times higher than the baseline levels. In the case of PAPI® and Hylene® W, small but statistically significant increases in airway resistance were recognized during sensitization, while animals exposed to MDI were within the baseline levels. In the recovery period, airway resistance of animals exposed to TDI decreased gradually and returned to baseline levels. In contrast, animals exposed to the other isocyanates returned to within baseline levels after a short period of time. After a single challenge exposure, the airway resistance increased abruptly in the animals exposed to TDI, while animals exposed to PAPI® showed a slight increase in the airway resistance. However, animals exposed to MDI and Hylene® W did not produce any statistically significant increase in airway resistance.

RESULTS (Continued)

Passive Cutaneous Anaphylaxis (PCA) Reaction

The results of cross sensitization are summarized in Table II. The guinea pigs sensitized with TDI antisera showed positive reactions, while other isocyanates failed to elicit the PCA reaction (Fig. 3). There were no cross sensitization reactions between TDI and the other isocyanates (Fig.12-17).

The sensitivity of reaction with TDI was not precisely equal in sensitized areas of the skin. The anterior dorsal skin and near the midline revealed clear-cut blue spots. The lateral skin showed somewhat more diffuse coloration. The most intense coloration was obtained in the nuchal skin, whereas caudal skin revealed less coloration. When 0.1 ml of TDI in dioxane was injected intradermally as the challenge dose, the central portion of the injection sites exhibited yellowish-white spots due to necrosis, and blue coloration developed around the necrotic spots. However, topical skin application with the challenge dose did not cause any necrosis and developed strong uniform blue spots. Positive reactions appeared within three minutes after challenge and reached maximum intensity within 15 minutes. Subsequently, the blue spots faded gradually, but a faint trace of blue color was still recognized 48 hours after the challenge.

Gel Diffusion Test

The undiluted antisera from a group of guinea pigs which were sensitized by aerosol exposure with four isocyanates were tested. In addition, antisera from other groups of rabbits and guinea pigs immunized by intramuscular injection with Freund's adjuvant or topical skin application were diffused on micro-Ouchterlony slides against TDI-BSA antigen or 1% solution of four different isocyanates in dioxane. Precipitin line did not form in any of the gel diffusion tests using different combinations of the antigens and antisera.

Pathology of Skin

Grossly, irregular-shaped blue spots were found on the cutaneous surface of the reaction sites, and the subcutaneous tissue was severely edematous, gelatinous in appearance and tinged with blue dye coloration. The sites which were injected with the control sera and challenged by the isocyanates revealed a slight polymorphonuclear leucocytic infiltration, especially around the perivascular areas of the dermis, the fibrous septa of adipose tissue and interstitial tissue of the muscle. The sites that were sensitized with TDI antisera and challenged by TDI exhibited prominent leucostasis and leucodiapedesis with edema in the subcutaneous tissue (Fig. 4). There were large accumulations of PMN leucocytes in the fibrous septa between the subcutaneous fat lobules and interstitial tissue of the muscle (Fig. 5). After 24 hours post-challenge, the number of PMN leucocytes and edema decreased markedly and was gradually replaced by lymphocytes and monocytes. The sites which were sensitized with other isocyanates and challenged by corresponding isocyanates or cross-challenged by TDI revealed a similar tissue reaction as observed at the control sites.

RESULTS (Continued)

Pathology of Lung

TDI Exposure

The superficial cells of the air passages were necrotic and partially sloughed off but rarely extended into the smooth muscle of the bronchial tree. The remaining epithelium showed hyperplasia and hyperactivity of the mucous secretion. Most of the air passages were filled with mucinous or fibrinous exudate which contained predominently PMN leucocytes, desquamated epithelial cells and a few round cells (Fig. 6). Some bronchiolar lumina were obliterated by mural polypoid protrusions with inflammatory exudate forming crescent-like passages. In a few severely damaged bronchioles, the lumen was almost completely obliterated by granulomatous tissue which was formed by organizing tissue leaving a slit-like space between the epithelium and the intraluminal granulation mass. The submucosa of the trachea and large bronchi was swollen and infiltrated by PMN leucocytes and round cells. The small bronchi and bronchioles were cuffed predominently with lymphocytes, plasma cells, monocytes and PMN leucocytes. The inflammatory reaction was confined sharply to peribronchial or peribronchiolar areas.

PAPI® Exposure

The air passages and alveolar response to PAPI® were basically similar to those of TDI. In contrast to TDI, PAPI® did not produce prominent acute inflammatory exudate and mucus in the air passages (Fig. 7). Inhaled PAPI® was readily recognized as minute droplets or as linear precipitate on the epithelium of the air passages causing necrosis and obliterative bronchiolitis (Fig. 8). The precipitate was slightly birefringent under polarized microscopic examination. Pale yellow refractile droplets, ranging in size from 1 to 5μ in diameter, were found mainly in desquamated epithelial cells, superficial epithelium of the air passages, alveolar macrophages, and alveolar septal cells (Fig. 9). The epithelial regeneration and organizing exudate became more pronounced on the seventh day post-exposure.

MDI Exposure

Most of the air passages appeared to be normal. A few bronchial trees showed desquamation of superficial epithelium and epithelial regeneration (Fig. 10). In comparison with other isocyanates, damage of air passages was very mild, and obliterative bronchiolitis was not encountered.

Hylene® W Exposure

In spite of epithelial damage of the passages, only a negligible amount of acute inflammatory exudate was found within the lumen and no obliterative bronchiolitis developed. In contrast to other isocyanates, the epithelium of the large air passages revealed prominent hypermucous secreting activity and precipitated material was not recognized in the bronchial epithelium (Fig. 11). Superficial tracheal epithelium was desquamated and showed partial squamous metaplasia. After the seventh day post-challenge, the epithelium of the air passages was regenerated and intraluminal exudate was organized.

Note: Detailed pathology of lungs is described in Pathology Reports No. 64-70 and No. 26-71.

DISCUSSION

The question as to whether immunologic mechanisms are involved in the hazard of isocyanate exposures is far from settled and beckons new investigative approaches. Also, little information is available about cross sensitization problems among isocyanates.

Clinical symptoms of workers exposed to isocyanates have suggested the possibility involving immunologically mediated hypersensitivity. However, no concrete evidence for an immunological response to isocyanates among workers appears to have been reported. Sensitive workers exhibited an asthma-like response to minimal atmospheric concentration of isocyanates which failed to provoke any pulmonary response to non-sensitized people (48). [illegible] showing typical bronchial asthmatic symptoms frequently [illegible] eosinophila (10, 65, 69, 75). Previously sensitized workers [illegible] marked asthmatic signs within a few minutes after inhaling an [illegible]nt amount of isocyanates (8). A relatively long latent period between initial exposure and symptoms suggestive of sensitization has been observed among exposed workers (65, 69). Since normal workers revealed asthmatic symptoms following exposure to relatively high airborne isocyanate concentration for a short period of time, or to moderate concentration for a longer period (10, 75), it is difficult to distinguish by clinical symptoms whether the asthmatic reaction to the isocyanate exposure is caused by direct chemical irritation or by immunological hypersensitivity.

The reaction of allergic inhalation test can be divided into immediate, late or dual in terms of their speed in appearing and peripheral respiratory (allergic alveolitis) or bronchial allergic reaction (42). Immediate reactions are of rapid onset and begin within 10 minutes, reach a peak by 15 to 30 minutes, and resolve spontaneously within one to three hours. The mechanism of the immediate allergic reaction in the human lung has not been clearly defined, but it is postulated that bronchial obstruction is largely attributable to mediators, probably histamine, SER-A, bradykinin, serotonin and prostaglandins (9). Release of enzymes from leucocytes may be an important phenomenon in the late allergic reaction (13, 47). The lysosomal enzymes of the PMN leucocytes are the source of the mediators involved in the development of inflammation and local vasculitis (73). Those workers exposed to TDI usually responded to late allergic reaction and occasionally to immediate reaction.

Immediate reaction occurs in the airways and causes asthma without systematic features such as fever and leucocytosis. This may provoke a blood eosinophilia usually associated with immediate-type skin sensitivity (25). The immediate asthmatic reaction correlated with reaginic skin sensitizing antibodies that have recently been identified as IgE immunoglobulins (22, 74). In atopic subjects, the immediate reaction is mediated by the IgE antibodies, while in nonatopic subjects it may be due to IgG antibodies (52).

Late asthmatic reactions begin between 3 and 13 hours, usually between 4 and 6 hours after allergen inhalation. They progress to a maximum more slowly - within one hour or over several hours and are more prolonged, usually within 24 to 48 hours, but may last for several days. Their features include febrile attacks with PMN leucocytosis, asthma and peripheral respiratory reaction or as an asthmatic reaction in which the systemic features are less predictable.

DISCUSSION (Continued)

These late asthmatic reactions have been observed following the inhalation of a variety of particles including house dust (7, 29, 44), grass (19), ragweed pollen (46), Bacillus subtilis enzymes (54), cotton dust (70), wood dusts (66), birds (53), plicatic acid (17), and amino-ethanolamine (67). Close correlation to dual skin reactions and precipitating antibodies have not been found in the inhaled particles just mentioned. The late reaction with pyrexial and peripheral respiratory reaction has been reported in patients with farmer's lung (5), bagassosis (26, 28), malt worker's lung (18, 62), bird fancier's lung (27, 61), mushroom worker's lung (31) and fish-meal worker's lung (3). They were associated with precipitating antibodies, and in malt worker's lung and bird fancier's lung with late skin reaction. The precipitating antibodies involved in late asthmatic reactions were IgA, IgG, or IgM (22).

Scheel, et al. used PCA and gel diffusion techniques to demonstrate specific TDI antibodies in the sera of rabbits immunized by intravenous injection of TDI egg albumin conjugates (64). However, the possibility that the serum antibodies might be associated with the carrier protein cannot be ruled out. Conversely, Thompson and Scheel reported negative results in an attempt to intensify sensitivity to TDI exposure for pertussis-treated rats and to depress sensitivity for alloxan-treated rats (72). They suggested that the pulmonary response to TDI exposure was due to chemical irritation rather than immune reaction (72). The serum antibodies were demonstrated with MDI egg albumin conjugates by the PCA test using guinea pigs and the sera from humans exposed to MDI (35). However, no detailed information is provided in order to question whether the exposed subjects became sensitized. Direct skin tests of apparently sensitized humans with TDI itself have failed to show positive reaction (68, 69). Recently, in an attempt to demonstrate serum TDI antibodies in the sera from humans, TDI human serum albumin (HSA) conjugates have been used as test antigens and utilized the following techniques: lymphocyte transformation test, PCA test, Prausnitz-Küstner test (P-K test), passive hemagglutination test, leucocytes histamine release, and the gel diffusion test. Of these tests, only the lymphocyte transformation test revealed positive reaction, suggesting the presence of TDI antibodies, other tests produced negative results (2, 8). The lymphoblast transformation of lymphocytes in culture presented some evidence to support the possibility that asthmatic symptoms of humans exposed to TDI may be an immunological reaction mediated by lymphocytes.

The molecular structure of the isocyanates used in these experiments was quite different, the only common thing was the presence of isocyanate group(s) (NCO). Bruckner, et al. noted that the isocyanate group attached to various aliphatic and aromatic molecules was responsible for the chemical reaction as well as the biological effects of the isocyanate compounds (8). In our antigen preparation for gel diffusion, it was impossible to accurately estimate the number of TDI haptens per molecule of BSA, since TDI reacted with water. Probably TDI-BSA conjugates may not be the proper immunological valence to elicit a precipitin reaction.

DISCUSSION (Continued)

Several investigators have demonstrated that the human immunoglobulin class differs in its ability to sensitize animal skin. Human skin sensitizing antibodies (reagens) reside in a unique immunoglobulin class that has been designated as IgE (23, 30, 37). These antibodies also sensitize monkey skin and produce P-K and PCA reactions parallel to those found by direct test (24, 38, 39). In contrast, the capacity to passively sensitize guinea pig skin is limited to certain subclass of IgG (71). Human precipitating antibodies gave positive PCA reactions in guinea pigs but not in monkeys (11). In the case of respiratory allergy due to moth flies, antibody activity was demonstrated in the patient's serum by the direct skin test and passive transfer to human and monkey skin, but not to guinea pig skin (60). In these experiments, five workers who worked in a plant producing isocyanates [illegible] products and who had asthmatic symptoms were subjected to the PCA [illegible] the guinea pigs. The number of subjects was too small to draw a [illegible] explanation for the negative results. However, it cannot rule out the possibility that the negative results of the PCA test might be ascribable to the human immunoglobulins which involved the bronchial asthmatic symptoms but were unable to sensitize the guinea pig skin. Among the several factors that might account for failure to obtain positive results from the PCA test with the sera from guinea pigs exposed to MDI, PAPI®, and Hylene® W, low antibody titer was suspected because the guinea pigs were exposed to relatively low atmospheric concentrations of isocyanates for a short period of time. In order to clarify this question, anti-isocyanate hyperimmune sera, which were obtained by topical skin application with isocyanates or by intramuscular injection with isocyanates in incomplete Freund's adjuvant, were used for the PCA test. Guinea pig anti-TDI sera produced uniformly positive reactions of PCA, but negative results were obtained invariably with other anti-isocyanates sera.

It has been reported that 50% of the asymtomatic individuals inhaling organic dust antigens may develop serum precipitins to those materials without developing any features of a hypersensitivity pneumonitis (4, 6, 12, 20, 23, 55, 61, 63). In addition, precipitins and MDI serum antibodies against known antigens tend to diminish or disappear with cessation of acute disease activity (36, 55). In view of these facts, an immunological test should be used only as confirmatory evidence. Observation of workers after natural exposure to isocyanates must be considered to have a more significant value for screening hypersensitivity.

Several authors have reported decreases in ventilatory function that occurred during the asymptomatic period in subjects suspected of respiratory sensitization by TDI (36, 69, 65). Some evidence for a cumulative effect was suggested by measurements of forced expiratory volume in second (FEV_1) for one week (59). Subsequently, the presence of a cumulative effect of TDI on ventilatory capacity was confirmed after follow-up studies among these workers for six months to two years (57, 58). In this study, the slight elevation of airway resistance which occurred during sensitization with Hylene® W and PAPI® might be attributable to a direct irritative effect on the air passages. A similar slight ventilatory decrease was reported in non-sensitized humans with isocyanates following exposure to TDI and MDI (36). Several chemical compounds were known to cause bronchospasm as a result of direct irritation on the respiratory passages (21, 24, 49). Conjectured

DISCUSSION (Continued)

from the observation of the abrupt increase in airway resistance with TDI challenge, the presence of serum antibodies detected by the PCA test, and the prominent exudative changes, as well as plasma cell infiltration in the airways, TDI may be a potent chemical sensitizer causing asthmatic pulmonary response. However, it should be mentioned that further immunological studies are necessary to prove definitely whether or not the serum antibodies demonstrated by the PCA test are responsible for respiratory hypersensitivity. Interpretation of the slight elevation of the airway resistance in guinea pigs following PAPI® challenge was difficult because the magnitude of airway resistance was not as drastic as that seen in TDI challenge and because of the negative results with the PCA test. In view of the marked obliterative bronchiolitis, slight [illegible] resistance may be related to damaged air passages and direct [illegible]tion rather than immunological reaction. Based on atmospheric [illegible]bration of isocyanates in these experiments, it is improper to [illegible] the results of airway resistance and pulmonary lesions seen in TDI exposure with other isocyanates because animals were exposed to different atmospheric concentrations. However, in terms of aerosol sensitization, atmospheric concentrations of other isocyanates were high enough to induce immunological reaction, since animals succumbed to the exposure when the atmospheric concentration was raised in an attempt to obtain hyperimmune sera by aerosol exposure.

To clarify whether negative PCA reactions of isocyanates, other than TDI, were due to sensitization with lower atmospheric concentrations, rabbits and guinea pigs were hyperimmunized by intramuscular injection or topical skin application with the same amount of isocyanates. The only positive PCA test reaction was obtained from TDI antiserum. All other isocyanate antisera produced a negative reaction. From these results, one can rule out the possibility that the negative PCA test was related to lower sensitizing concentrations of the isocyanates. A possible explanation for the positive PCA reaction after TDI exposure is the relatively high concentration of NCO groups in TDI in comparison with the other isocyanates. Since the equivalent weight of TDI is approximately 70% of the other isocyanates tested, the guinea pigs in the TDI exposures received roughly 50% more NCO groups per weight of dose than for the less volatile isocyanates. There is a difference, therefore, of one to two orders of magnitude greater concentration of isocyanate functions in the TDI experiments vs. the other isocyanates.

Little is known regarding pulmonary response to immunological reaction. Liebow described the following pathological changes as criteria to allergic pneumonitis: extensive eosinophilic reactions, plasma cell infiltration, angitis or granulomatosis, and noncaseating granuloma such as sarcoidosis (41). The asthmatic patients exhibited gamma globulins IgA, G, and/or M deposits beneath the bronchial epithelium (14, 15, 68). In patients with asthma, recent immunohistochemical studies have shown nonspecific localization of IgE. It was impossible to distinguish the skin test positive individual from the skin test negative individual on the basis of the number of IgE-containing mononuclear cells in the bronchial section (14). The lung can be part of acute generalized allergic reactions

DISCUSSION (Continued)

characterized by exudation. The infiltration of PMN leucocytes occurred in the early stages of allergic reaction in farmer's lung (6). The mononuclear cells, plasma cells and lymphocytes appeared in the latter stage of the allergic reaction.

After the TDI challenge, the striking histological changes were heavy infiltration of PMN leucocytes in the airways superimposed upon changes of chronic obliterative bronchiolitis, and dense peribronchiolar cuffing with plasma cells. The chronic bronchiolitis was probably produced by consecutive sensitization exposure with TDI, and subsequently heavy PMN leucocytic infiltration was induced by challenge exposure. Abundant exudate, mainly PMN leucocytes and damge in the airways appeared to be partially responsible for the marked elevation of airway resistance.

KPL:ljm
April 16, 1974

TABLE I

Comparison of Airway Resistance in Guinea Pigs Sensitized cyanates

Average cm H_2O/Ml/Sec ± Standard Deviation

Isocyanate	Pre-Exposure Period	Sensitization Period	Recovery Period	Challenge Period
Control	0.5800 ± 0.06	0.4769 ± 0.03	0.5502 ± 0.05	0.4574 ± 0.07
PAPI	0.5335 ± 0.01	0.6438 ± 0.13	0.5453 ± 0.03	0.7503 ± 0.21
TDI	0.4899 ± 0.05	1.1609 ± 0.56	0.8180 ± 0.24	1.1693 ± 0.30
Hylene® W	0.5829 ± 0.10	0.7332 ± 0.16	0.5485 ± 0.05	0.4240 ± 0.05
MDI	0.4889 ± 0.06	0.4231 ± 0.10	-	0.4702 ± 0.08

TABLE II

Cross-Reactivity of Guinea Pigs Sensitized by Isocyanate Compounds

Test Compound Skin Application (Challenge)	Number of Guinea Pigs	Intradermal Injection Antisera (Sensitizer)				
		Control	TDI	PAPI®	MDI	Hylene® W
TDI	51	-	+	-	-	-
PAPI®	17	-	-	-	-	-
MDI	7	-	-	-	-	-
Hylene® W	9	-	-	-	-	-

Code: - = Negative

- = Slight degree of change.

TABLE III

Pulmonary Lesions of Guinea Pigs Exposed to Isocyanate Compounds

Pathological Lesions in Air Passages	Isocyanate Compounds			
	TDI	MDI	PAPI®	Hylene® W
Epithelial Desquamation	++	+	++	++
Intraluminal Inflammatory Exudate	+++	±	+	±
Epithelian Hyperplasia, Regeneration	++	±	++	++
Obliterative Bronchiolitis	++	-	++	-
Peribronchiolitis	+++	±	+	±
Precipitate	-	-	+++	-

Code: - = Negative.
± = Very slight degree of change.
+ = Slight degree of change.
++ = Moderate degree of change.
+++ = Marked degree of change.

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